PEG-YLATED PARENTERAL NANOEMULSIONS AS PROSPECTIVE CARRIERS FOR ENHANCED BRAIN DELIVERY WITH DIAZEPAM AS A MODEL DRUG – PHYSICOCHEMICAL CHARACTERISATION

Đoković J. [1], Konkel M. [2], Mitrović J. [1], Savic S.M. [3], Watrobska-Swietlikowska, D. [2], Cekić N. [3], Savić S.D. [1]

[1] Department of Pharmaceutical Technology and Cosmetology, University of Belgrade – Faculty of Pharmacy, Vojvode Stepe 450, Belgrade, Serbia

[2] Department of Pharmaceutical Technology, Medical University of Gdansk, Hallera 107, 80-416 Gdansk, Poland

[3] Faculty of Technology, University of Niš, Leskovac 16000, Serbia

e-mail of corresponding author: snezana.savic@pharmacy.bg.ac.rs

ABSTRACT

Parenteral nanoemulsions are regarded as biocompatible drug delivery systems for lypophilic drugs. When it comes to delivering actives to the brain as a target site, prolonged circulation time is desirable. The objective of this study was to conduct physicochemical characterization of PEGylated nanoemulsions as prospective carriers for enhanced brain delivery, using diazepam as a model active substance for brain targeting. Nanoemulsions were prepared by high pressure homogenization method and characterized regarding droplet size, zeta potential, pH, conductivity, viscosity and in vitro release profile. PEG2000-DSPE and PEG5000-DPPE were used for PEGylation. All the formulations were autoclaved and stored at room temperature. After 2 months there were no significant changes in physicochemical parameters in autoclaved formulations which rendered them as good potential templates to incorporate drugs for brain targeted delivery.

Keywords: PEGylated nanoemulsions, diazepam, physicochemical characterization, in vitro release

INTRODUCTION

Nanoemulsions are regarded as biocompatible drug delivery systems which are especially beneficial for parenteral application of water insoluble actives. It is considered that the oil droplets are rapidly removed from the circulation by the components of the mononuclear phagocytic system (MPS) [Hormann, 2016]. When it comes to brain as a target site, prolonged circulation time of oil droplets is beneficial as it allows more time for an active to reach and cross the blood brain barrier. One of the strategies used to prolong the circulation time of parenteral nanoemulsions is coating the surface of oil droplets with polyethyenglicol (PEG) chains. PEG chains provide protection against detections by opsonins from plasma and MPS removal from the circulation by increasing the surface hydrophilicity [Kandadi, 2011]. Diazepam is available on the market as nanoemulsion preparation (Diazelmus, Kabi-Pharmacia, Sweden) and is commonly used benzodiazepam [Đorđević, 2013]. The aim of this study was to develop and characterize PEGylated parenteral nanoemulsions as possible carriers for prospective delivery of actives to the brain, using diazepam as a model active substance.

RESEARCH CONCEPT

Nanoemulsions (non-PEGylated - NPEG and PEGylated) were prepared by high pressure homogenization method, autoclaved and characterized regarding droplet size, zeta potential, pH and conductivity both initially and after 2 months of storage at room temperature. Additionally, viscosity measurements and *in vitro* release study of the formulations were performed.

1. Nanoemulsion preparation and sterilization

Diazepam loaded nanoemulsions (2mg/g) were prepared using high pressure homogenization method

https://doi.org/10.24355/dbbs.084-202001221435-0

at room temperature. Water and oil phase were prepared separately. Aqueous phase (glycerol, polysorbate 80, highly purified water and 0,1 M NaOH) was added to oil phase (soybean oil, medium chain triglycerides, soybean lecithin, butylhydroxytoluene and diazepam) and homogenized using rotor stator homogenizer (IKA Ultra-Turrax® T25 digital) at 8000 rpm for 3 minutes. Formulations were further homogenized by high-pressure homogenizer (EmulsiFlex-C3, Avestin Inc., Canada) for 9 cycles at 500 bar. PEGylation agents used were PEGylated phospholipids: PEG2000-DSPE (P2) or PEG5000-DPPE (P5), which were added to the aqueous phase at concentration of 0,1% or 0,3% (P2_0.1%, P2_0.3% P5_0.1%, and P5_0.3%). All formulations were sterilized in autoclave at 121°C for 15 minutes.

2. Size and zeta potential measurements

Formulations were characterized regarding the mean droplet size (intensity weighed mean diameter, Z-Ave), droplet size distribution - polydispersity index (PDI) and zeta potential (ZP) by Zetasizer Nano ZS90 (Malvern Instruments Ltd., Worcestershire). In order to confirm the size measurements and check for larger droplets presence laser diffraction measurements were performed using Beckman Coulter LS 13 320 (Beckman Coulter, Inc., Brea, California).

3. Conductivity, pH and viscosity

Conductivity and pH measurements were performed to test the formulations' stability after sterilization and during storage as well as suitability for parenteral application. Viscosity measurements were conducted in order to assess the formulations' syringeability and suitability for parenteral application.

4. *In vitro* release studies

The drug release was studied by dialysis bag method using cellulose membrane with molecular weight cut off of 12000. Dialysis bags with 2 ml of the formulations were placed in 200 ml of the dissolution medium - phosphate buffer pH 7,4 (USP) : methanol = 80:20 (*v/v* %). Samples were drown after 0.083, 0.167, 0.333, 0.67, 1, 2, 4, 8, 12 and 24 hours and analyzed for diazepam content by spectrophotometer at 230 nm. The release profiles were analyzed with different kinetic models: zero order, first order, Higuchi, Baker-Lonsdale, Korsmeyer-Peppas and Hixon-Crowel models (DDSolver packet for Microsoft Excel apication).

RESULTS

The size measurements preformed initially and after 2 months of storage showed that Z-ave was in the range of 180 nm to 220 nm, with PDI below 0,2 for both non-autoclaved and autoclaved samples (**Figure 1**.). Laser diffraction measurements showed that d_{100} was below 1 µm for all samples.

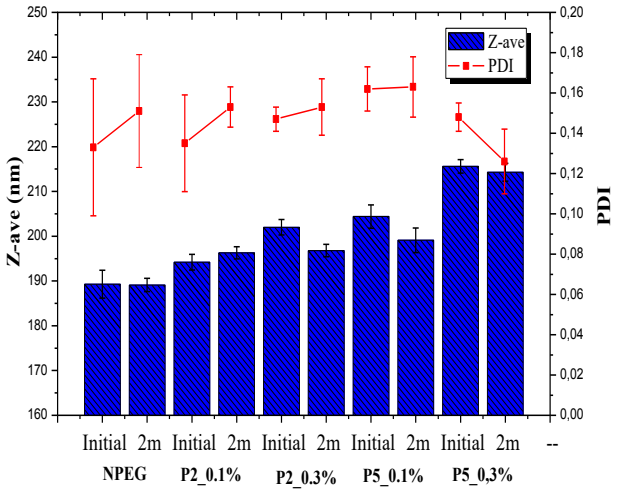

Figure 1. Z-ave and PDI results of the non-autoclaved formulations

Zeta potential for all the samples was between -30 and -50 mV (**Figure 2)**. Non-autoclaved samples had significant changes in pH (decreasing from around 7 to about 5 within 2 months) and conductivity (increasing from around 100 µS/cm to around 400 µS/cm). As for the autoclaved samples, pH and conductivity remained stable at around 7 and 100 µS/cm, respectively. Viscosity was around 5 mPa*s for all samples.

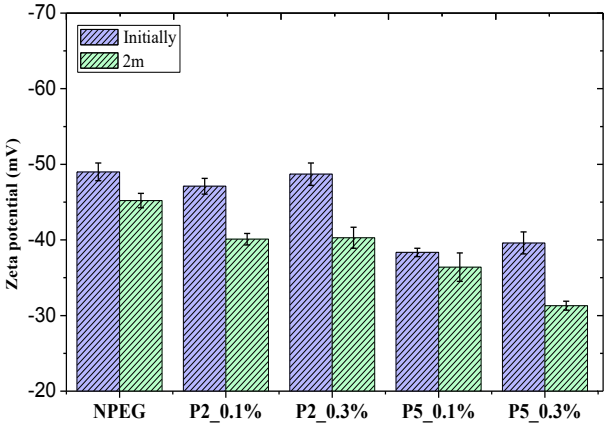

Figure 2. Zeta potential for the non-autoclaved formulations.

https://doi.org/10.24355/dbbs.084-202001221435-0

The results from in vitro release study are shown on **Figure 3.**

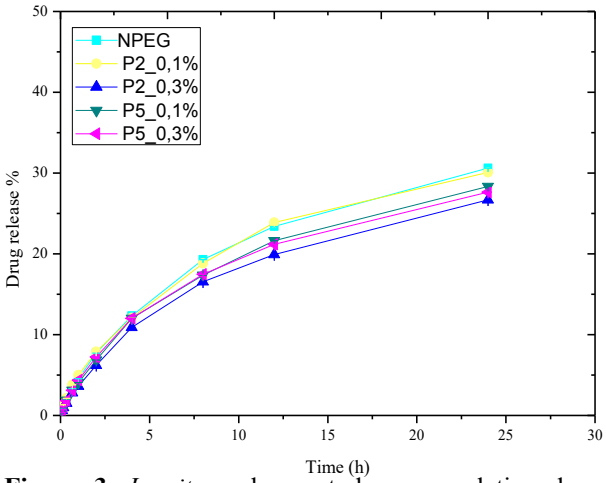

Figure 3. *In vitro* release study – cumulative drug release

DISCUSSION

All the PEGylated formulations had higher droplet size comparing to the non-PEGylated formulation and Z-ave increased with increasing concentration of PEGylated phospholipids. Droplet size was higher when using PEG5000-DPPE compared to PEG2000-DSPE, which can be explained with the longer of PEG chains in PEG5000-DPPE formulations. Nevertheless, the Z-ave was below 500 nm for all the formulations, with no droplets larger than 1 μm detected, which is concordant with USP requirements. PDI remained below 0.2 showing narrow size distribution. Given that the formulations are stabilized with soybean lecithin, zeta potential values at around -40 mV were expected. During storage and autoclaving phospholipids and oils can succumb to hydrolysis, which in turn leads to increased negative zeta potential value. This could be perceived as beneficial given that the higher zeta potential values indicate better stability, but in this case it is a consequence of free fatty acid liberation, which leads to pH value decrease, which in turn promotes further degradation and destabilization in nanoemulsions [Klang, 2011]. Interestingly, in obtained autoclaved nanoemulsions showed better stability in terms of maintaining pH and conductivity values compared to the unsterilized formulations, which requires further insight . Lower absolute zeta potential values can been detected in formulations containing PEG5000-DPPE versus the non-PEGylated and the formulations containing PEG2000-DSPE, probably due to longer PEG chains increasing hydrophilicity on the surface. [Kandadi, 2011]. Interestingly, it appeared that the autoclaved samples showed better stability compared to the non-autoclaved samples regarding pH and conductivity measurements. Low viscosity of the formulations rendered them safe for parenteral use and showed that adding PEGylated phospholipids did not significantly increase the formulations' viscosity.

In the *in vitro* release study it could be observed that the highest release of diazepam was from the non-PEGylated formulation (Figure 3). That release profile was similar with the formulation containing 0,1 % PEG2000-DSPE, suggesting that the used concentration was too low to coat the nanoemulsion droplet surface and slow down the release. The slowest release was observed when using 0,3 % PEG2000-DSPE compared to both the non-PEGylated formulation and the formulations containing PEG5000-DPPE, which could be explained by PEG2000-DSPE forming more rigid packing at the interface comparing to DPPE chains, therefore causing slower release of the incorporated diazepam. This was corroborated by the fact that the Higuchi model, which describes drug release as a diffusion based process founded in the Fick's law was the best fit to describe the profiles (highest R^2, R_{adj}^2 and the lowest AIC - Akaike Information Criterion) [Costa, 2001]. Higuchi dissolution constants were lower for the formulations containing PEGylated phospholipids which indicated that they slow down the diffusion of the diazepam from the droplets.

CONCLUSIONS

Initial physicochemical characterization suggested that investigated nanoemulsions were appropriate for parenteral application. Release profiles showed that the PEGylated formulations could delay the release of the incorporated drugs, which could be beneficial when prolonged release of the active is required. They could be considered as prospective template carriers for the water insoluble actives when prolonged circulation time is desired, for example for brain targeted delivery. However, further investigations are necessary in order to prove pharmacokinetic advantages *in vivo*, like pharmacokinetic study in animal models.

ACKNOWLEDGMENT

This work was supported by the Ministry of Education, Science and Technological development, Republic of Serbia, within the framework of the project TR34031 as well as CEEPUS project CIII-RS-1113-02-1819

https://doi.org/10.24355/dbbs.084-202001221435-0

Central European Knowledge Alliance for Teaching, Learning and Research in Pharmaceutical Technology (CEKA PharmTech).

REFERENCES

[Hormann, 2016] Hormann K., Zimemer A., 2016, Drug delivery and drug targeting with parenteral lipid nanoemulsions-a review, *J. Control. Release*, Vol. 223, pp. 85-98.

[Kandadi, 2011] Kandadi P., Afzal S. M., Goparaboina S.and Veerabrahma K. ,2011, Brain specific delivery of pegylated indinavir submicron lipid emulsions, *Eur. J. Pharm. Sci.*, Vol 42,pp. 423-432.

[Đorđević, 2013] Đorđević S., Radulović T., Cekić N., Ranđelović D., Savić M., Krajišnik D., Milić J., Savić S., 2013, Experimental design in formulation of diazepam nanoemulsions: physicochemical and pharmacokinetic performances, *J. Pharm. Sci*., Vol. 102, pp. 4159-4172.

[Klang, 2011] Klang, V., Valenta, C., 2011. Lecithin–based nanoemulsions. *J. Drug Del. Sci. Tech*. Vol. 21,pp. 55–76

[Costa, 2001] Costa, P., Sousa Lobo, J.M., 2001. Modeling And Comparison Of Dissolution Profiles. *Eur. J. Pharm. Sci.* Vol. 13, pp.123–133.

https://doi.org/10.24355/dbbs.084-202001221435-0